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14. ABSTRACT

The study aim is to investigate specific mechanisms of adverse effects related to RBC storage age in critically ill patients. To date we have enrolled 79 patients at the three clinical sites in Canada. Samples have been collected, processed, and shipped from the sites in Quebec, Canada to the repository at Blood Systems Research Institute (BSRI) in San Francisco, California. The repository consists of plasma, PBMCs and whole blood samples. The testing of patient samples for coagulation parameters, microparticles and microchimerism has begun. Results from these tests will be correlated upon study completion.

15. SUBJECT TERMS
RBC storage age; subject enrollment; IRB approval; repository; Microparticles; Coagulation; Microchimeris

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INTRODUCTION

Combat casualties are specifically at risk of adverse effects resulting from the use of RBCs of increased storage age. A large multicenter randomized controlled trial in 30 Canadian centers of 2500 critically ill patients called the Age of Blood Evaluation (ABLE) trial has been funded. In this trial of critically ill patients, which includes patients with traumatic injuries, study groups will be randomized to either RBCs of < 8 days storage time or standard RBC storage time (mean 21 days). The primary outcome of this trial is 90 day mortality. Secondary outcomes include severity of multiple organ dysfunction syndrome, serious thrombotic events and nosocomial infections, and ICU and hospital length of stay. Prospective clinical studies investigating the mechanisms and clinical outcomes associated with increased or decreased RBC storage age in critically ill patients including traumatic injury have not been performed. The ABLE study presents a unique and probably one-time opportunity to investigate mechanisms in the context of clinical outcomes for well-characterized study groups. This study is designed to determine specific mechanisms of adverse effects related to the RBC storage age in transfused critically ill patients enrolled in the ABLE study. This ancillary study will specifically determine if the RBC unit storage time affects patient's immune function, inflammation, coagulation, microparticle concentrations and microchimerism.

Hypotheses

1.) Increased storage time of transfused RBC units will affect both inflammation and coagulation factors in critically ill patients and these parameters will be positively associated with measured clinical endpoints including increased morbidity (sepsis, serious thrombotic events, multi-organ failure) and mortality.

Aims

2.) To determine how RBC unit storage time affects inflammation and coagulation in critically ill patients, how these effects change over time after transfusion and if these parameters correlate with clinical outcomes.

    1a. Measure the levels of pro-and anti-inflammatory cytokines and coagulation factors in serum from transfused subjects longitudinally using multiplex assays (high and standard sensitivity).

    1b. Quantify levels of markers associated with cardiovascular disease including cellular adhesion molecules and growth factors using multiplex bead-based assays.

    1c. Correlate patterns of cytokine and inflammatory marker secretion and measures of coagulation with receipt of blood stored for short vs. long periods.

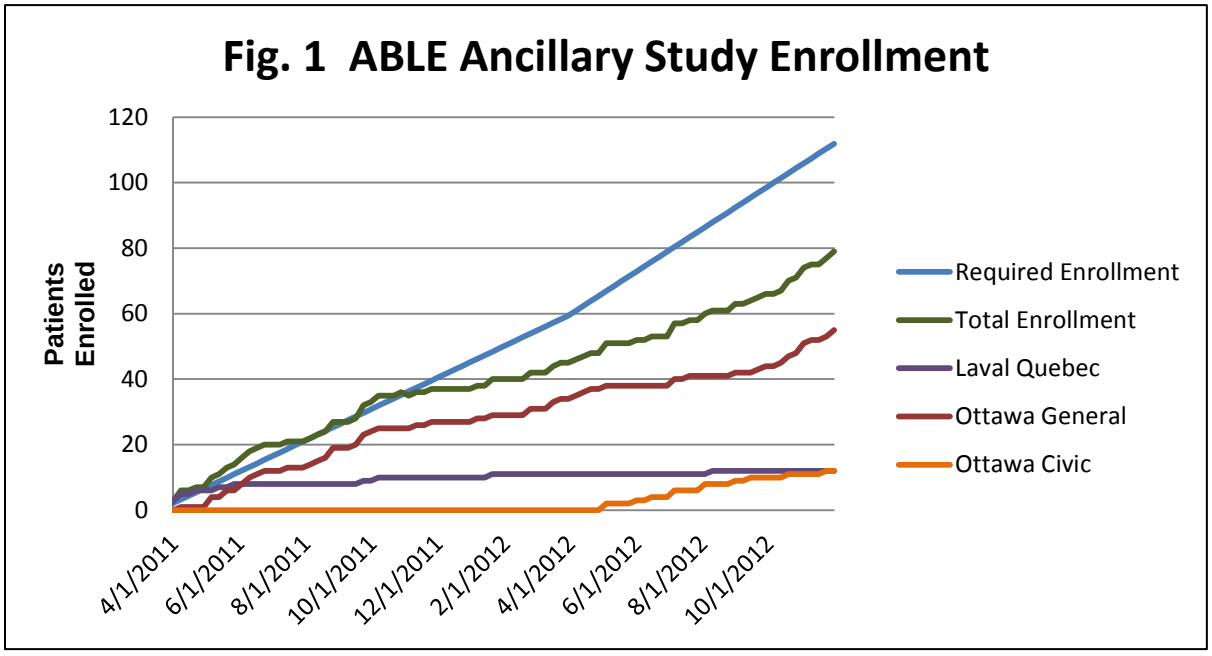
    1d. Correlate patterns of cytokine and inflammatory marker secretion and coagulation with all clinical outcomes.

3.) To develop a patient sample repository for future analysis of additional effects of RBC storage age in critically ill patients.

BODY

Since the last annual report, we have continued to enroll patients and collect samples at all sites. As of November 30th 2012, we have enrolled a total of 79 patients in this study. In the first quarter, enrollment rates declined as Laval Hospital stopped enrollment due to shortage of staff. However, they started to enroll patients again in the second quarter. In the meantime, Ottawa Civic Hospital began enrollment as they were provided with necessary study equipment in February of this year. This boosted the rate of enrollment and allowed us to catch up to the required enrollment rate. We are in the process of recruiting additional sites in order to meet the study enrollment goals.

Figure 1 below shows total enrollment versus expected enrollment (as of November 30, 2012). Graph also shows actual enrollment for each of the sites.



Evaluable samples have been collected, processed and shipped from the clinical sites to BSRI. These samples are being stored at BSRI. Cytokine analysis will be performed in batches in order to avoid variability of test results due to testing procedures and/or reagents used. We have started performing coagulation testing in batches (see Fig. 2 for representative markers). However these results can neither be correlated neither with patient status of receiving old or new blood nor with clinical outcomes, as that information will only be available once the study is completed. Similarly, MP counts have been obtained in the first donors, but again we are blinded to the clinical status of the study subjects, making interpretation of the data difficult.

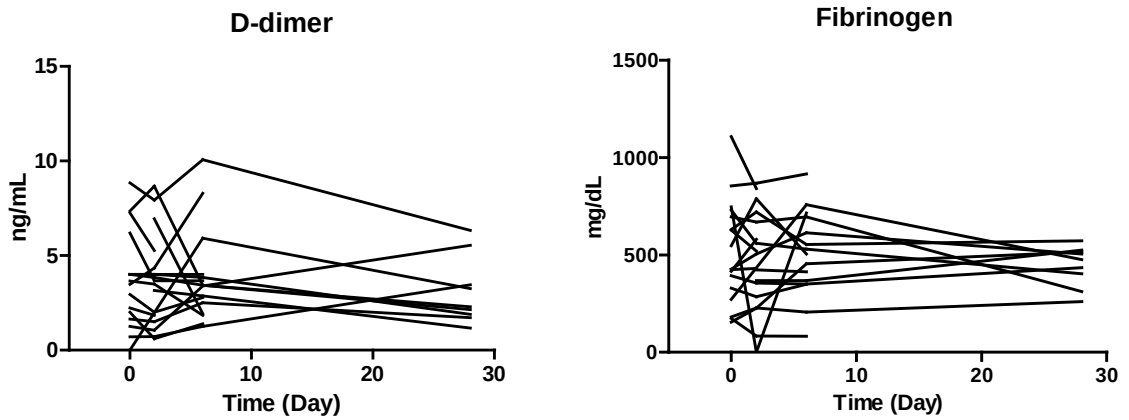


Fig. 2 Evolution of coagulation markers after enrollment in the first 20 subjects. Samples were tested at Days 0, 2, 6, and 28. Results demonstrated relative stability of D-dimer and fibrinogen levels in these subjects over time from enrollment.

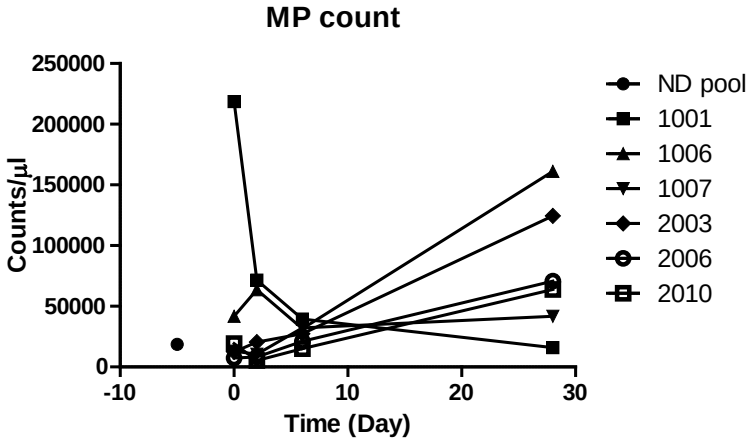


Fig. 3 Evolution of microparticle counts after enrollment in the first 6 subjects. Samples were tested at Days 0, 2, 6, and 28. Control MPs were tested using MPs pooled from 3 normal donors. Results show early elevated MPs in some subjects, with late elevations in others compared to controls.

KEY RESEARCH ACCOMPLISHMENTS

- We continue to enroll patients and collect samples at the clinical sites in Canada
- The samples are being processed, shipped and stored.
- To date, 79 patients have been enrolled.
- We have completed coagulation testing on 60 samples. A third batch of 30 samples is currently being analyzed.
- We have completed DNA typing for Day 0 samples for 10 patients.
- We have completed microparticle testing on all samples from 6 patients.

REPORTABLE OUTCOMES

We have continued building a repository of plasma, PBMCs and whole blood samples. We have begun coagulation, micro particle and microchimerism testing.

CONCLUSION

The ABLE ancillary study has continued to enroll patients and collect samples. All sites are currently active and enrolling patients. Testing will accelerate in the coming year as we have a critical mass of samples to perform batch testing for the immunology and coagulation assays. Microchimerism testing will also proceed as samples are acquired.

REFERENCES

None

APPENDICES

None